The protease fibroblast activation protein [FAP] as a biomarker and therapeutic target in chronic liver injury

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Fibroblast Activation Protein (FAP) - similarity to Dipeptidyl Peptidase 4 (DPP4)

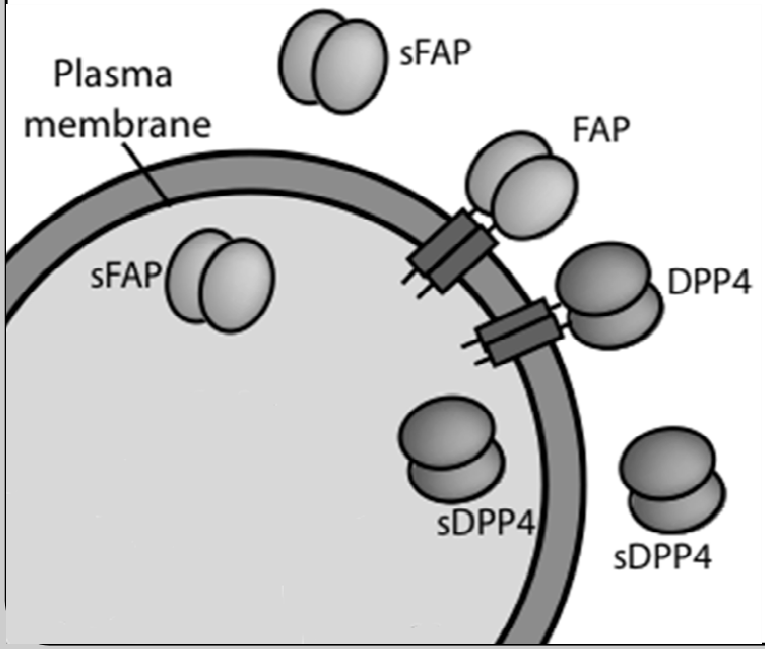
Protease Activity:

Dipeptidyl Peptidase

N-term X - P - X - X - X - …. C-term

Endopeptidase

N-term…. - X - X - G - P - X - X - …. C-term



DPP4 roles:

Type 2 Diabetes
Tumor growth
Liver fibrosis & fatty liver

FAP roles:

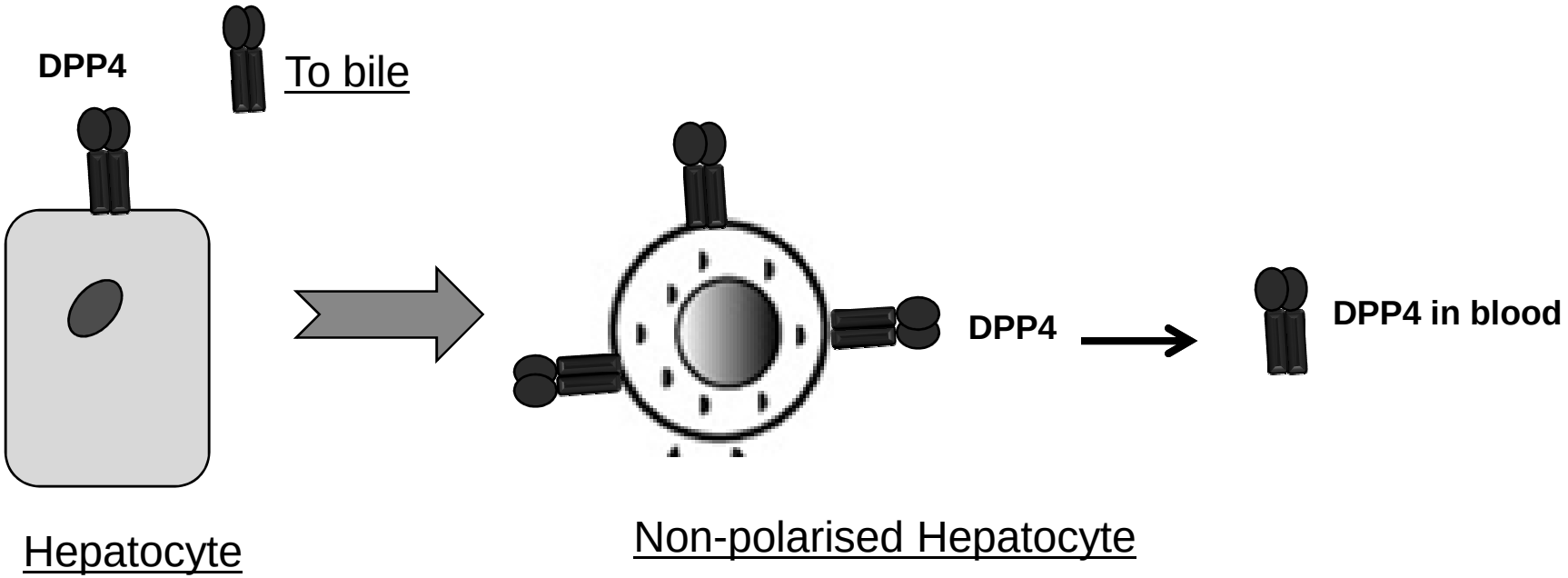
Fibrinolysis
Tumor growth
Atherosclerosis
Liver fibrosis & fatty liver

Gorrell 2005 *Clinical Science* **108**: 277
Hamson, Gorrell 2014 *Proteomics Clin Appl* **8**(6): 454

DPP4 in Chronic liver injury

Healthy liver

Injured liver



DPP4 increases in epithelial cells and lymphocytes

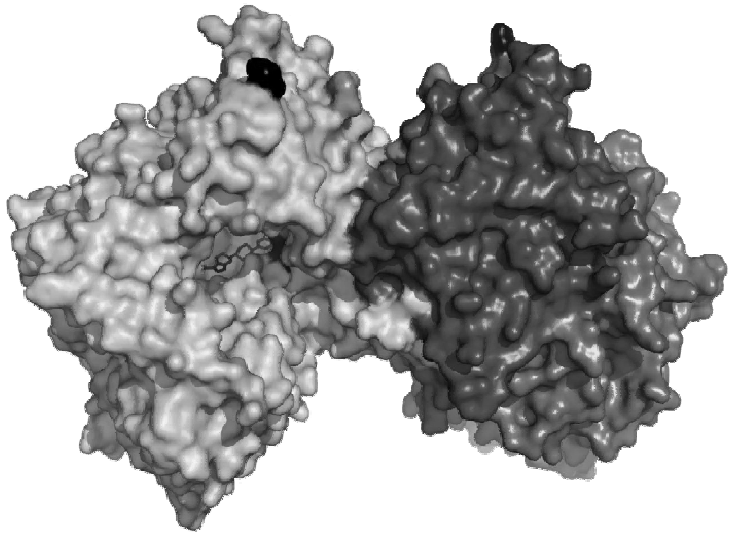
DPP4 in Chronic Liver Injury

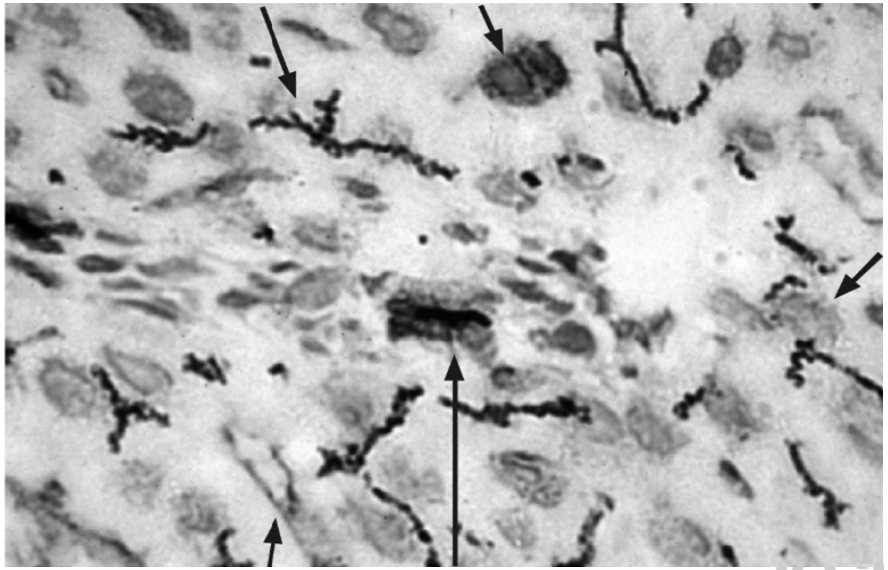
- DPP4 is ubiquitous. But liver is a large organ…

- DPP4 expression increases in fibrosis and cirrhosis: Both in liver and blood. [Williams K, Gorrell, Zekry, Twigg et al 2014 *J. Diabetes* in press; doi 10.1111/1753-0407.12237]

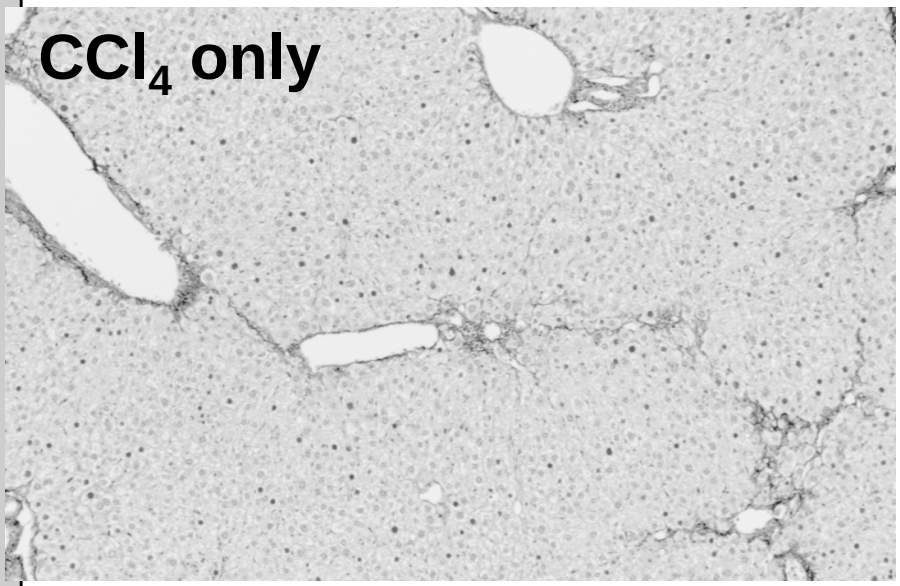
- Preclinically, DPP4 inhibition lessens steatosis.

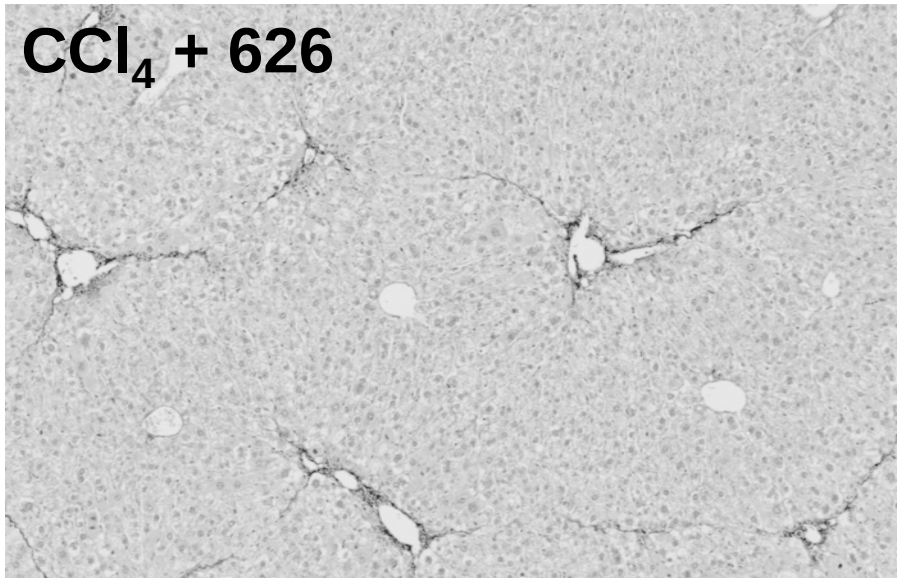
Review: Itou 2013 *World J Gastro* **19**: 2298



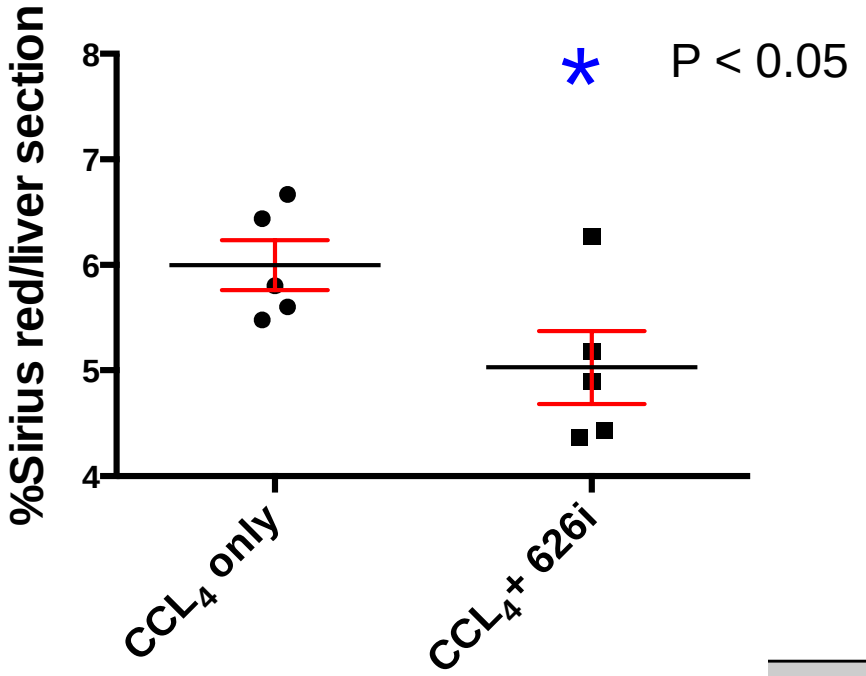


DPP4 inhibition can lessen liver fibrosis



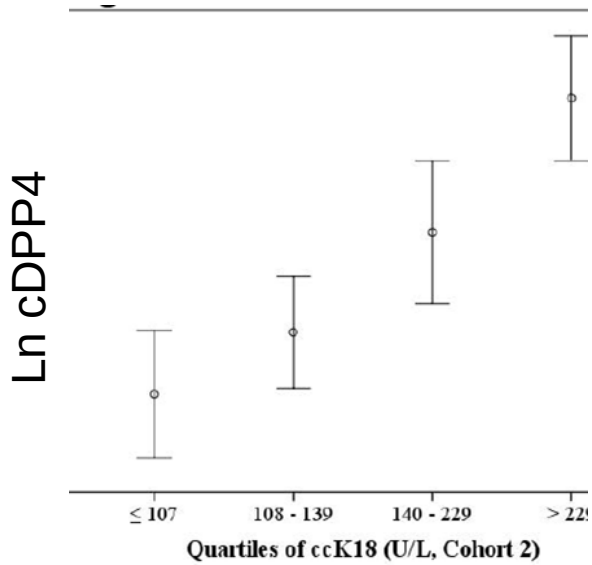


4 weeks of CCl_4
With DPP4 inhibition
[MK626 ; MSD]

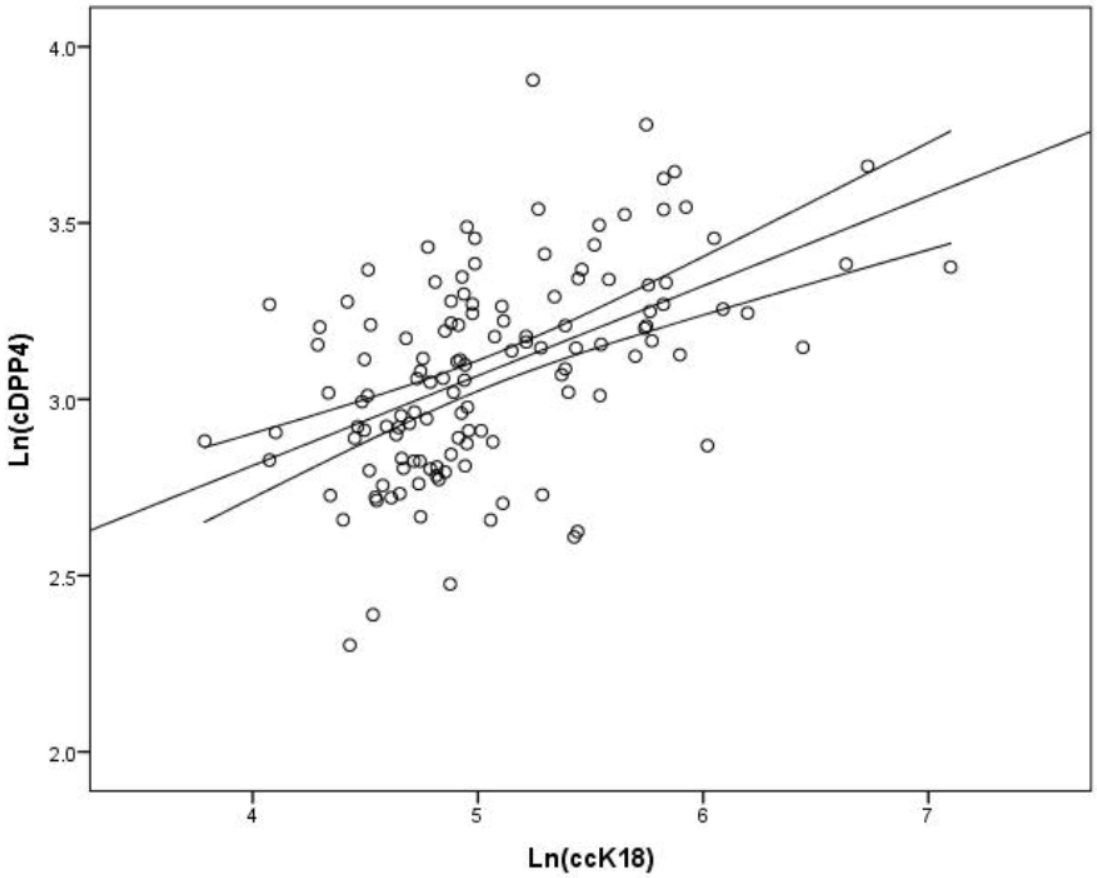


Sirius red staining in liver

DPP4 as a biomarker



Williams K, Gorrell, Zekry, Twigg et al 2014 *J. Diabetes* in press; doi 10.1111/1753-0407.12237

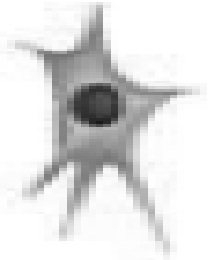


Fibroblast activation protein: [FAP]: Unique Expression

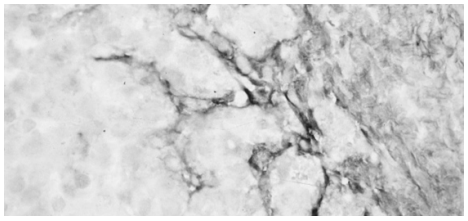
- LOW expression in normal resting adult tissue

- <u>Tissue Remodelling</u>
  - Embryogenesis
  - Wound healing

- <u>Activated Fibroblasts</u>
  - Epithelial tumours
  - Arthritis
  - Atherosclerosis
  - Liver and lung fibrosis



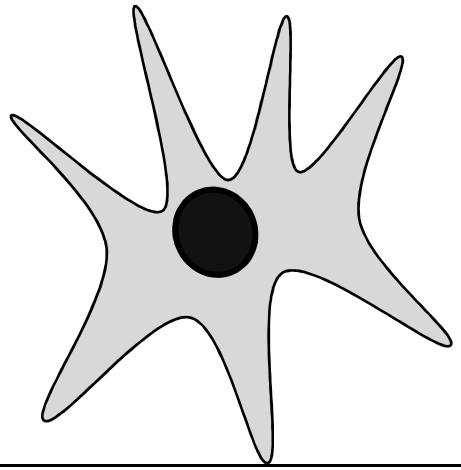
  - Activated myofibroblasts
  - Activated Stellate cells



FAP in human liver

FAP in Chronic liver injury

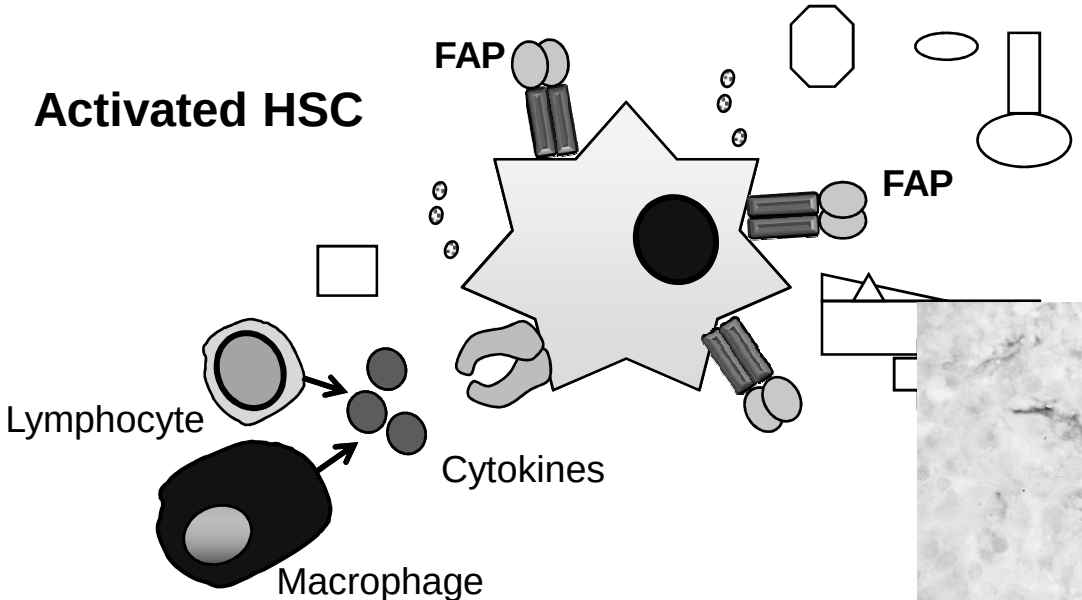
Quiescent Hepatic Stellate cell (HSC)



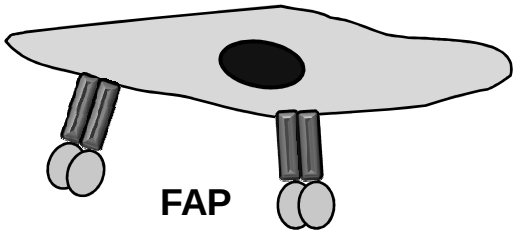
FAP very low

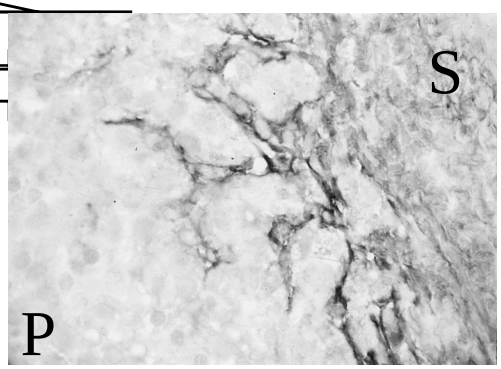
FAP high on HSC and myofibroblast

Activated HSC



myofibroblast





FAP correlates with fibrosis severity
(Levy, 2002)

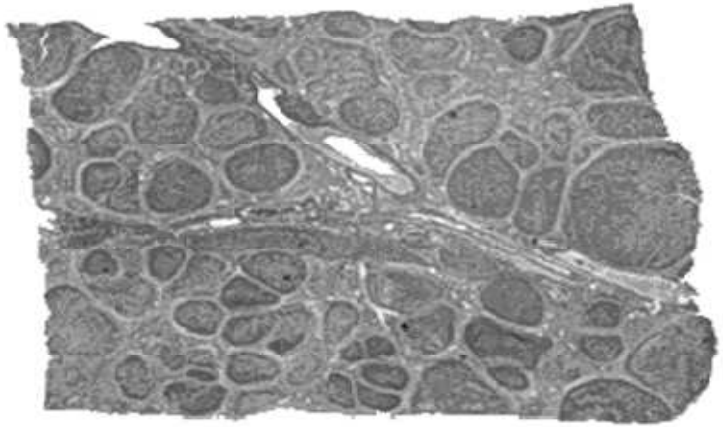
FAP in human liver is pro-fibrotic

- Expressed by **activated** hepatic stellate cells [HSC] and myofibroblasts in chronic liver injury (Levy Gorrell *et al* 1999 *Hepatology*)

- Intensity of FAP expression correlates with **fibrosis** severity (Levy, Gorrell 2002 *Liver Internat*)

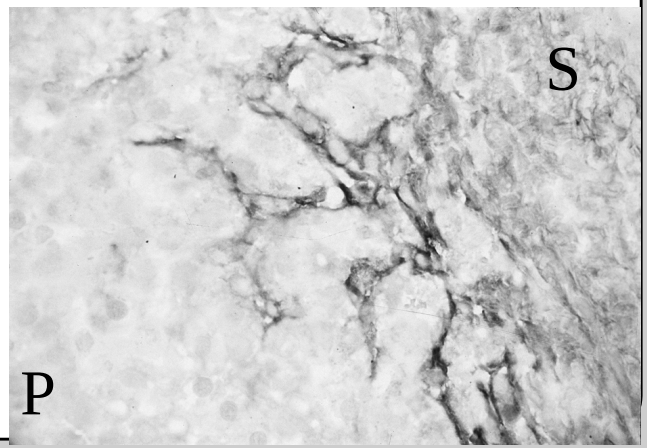
- FAP expression is stimulated by TGFβ and retinoic acid.
- **Gelatinase** (collagen-I) and **DPP** activities in liver (Park 1999; Levy, Gorrell 1999 *Hepatology*)
- Collagen cleavage by FAP is enhanced by MMP1 cleavage.
- Fibrinolysis inhibition by cutting human α2-antiplasmin (*Lee 2012 J Thromb Haemost*)

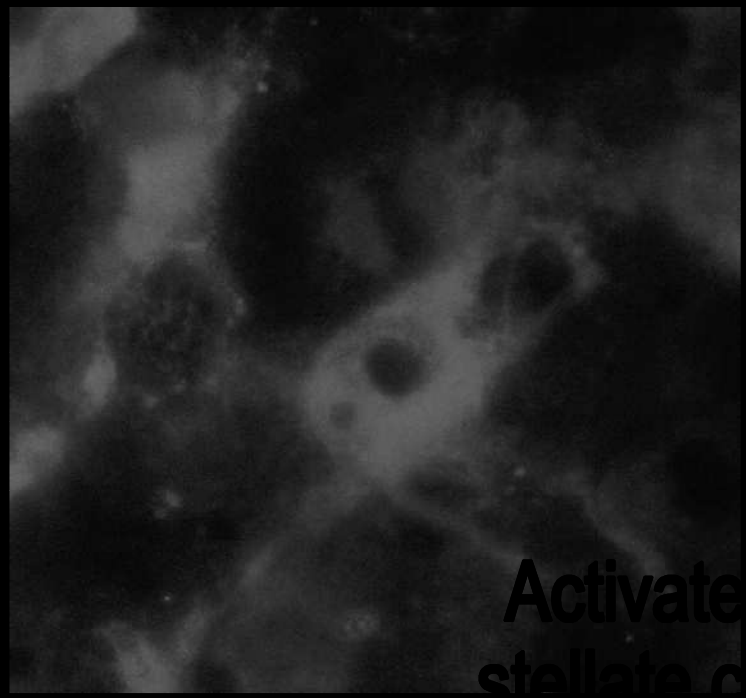
- See: Gorrell & Park 2013 Fibroblast activation protein alpha. In *Handbook of Proteolytic Enzymes 3rd Edition*.
- See: Hamson, … Gorrell 2014 *Proteomics Clin Appl* **8**(6): 454.

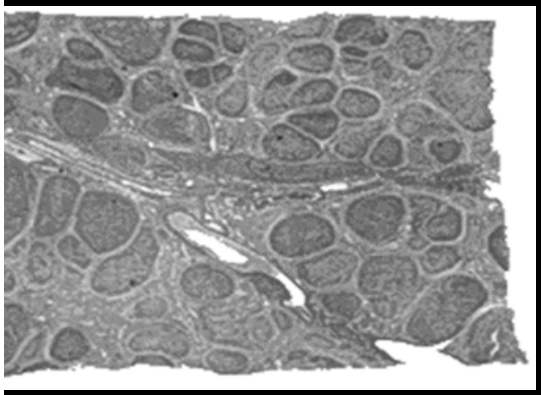


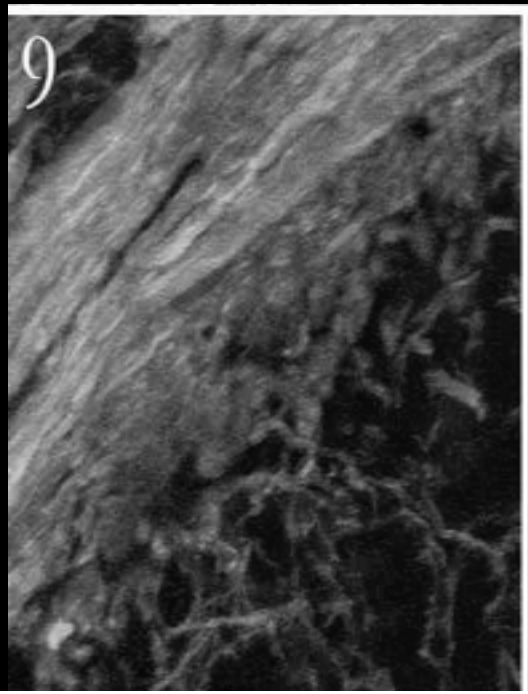
FAP in human cirrhotic liver

(Wang, Gorrell 2005 Hepatology)




Activate

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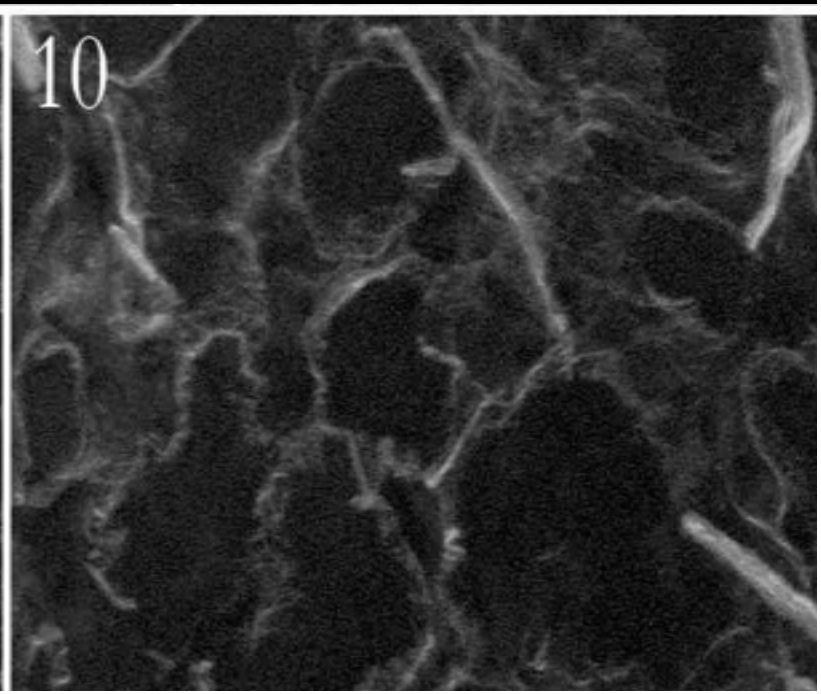
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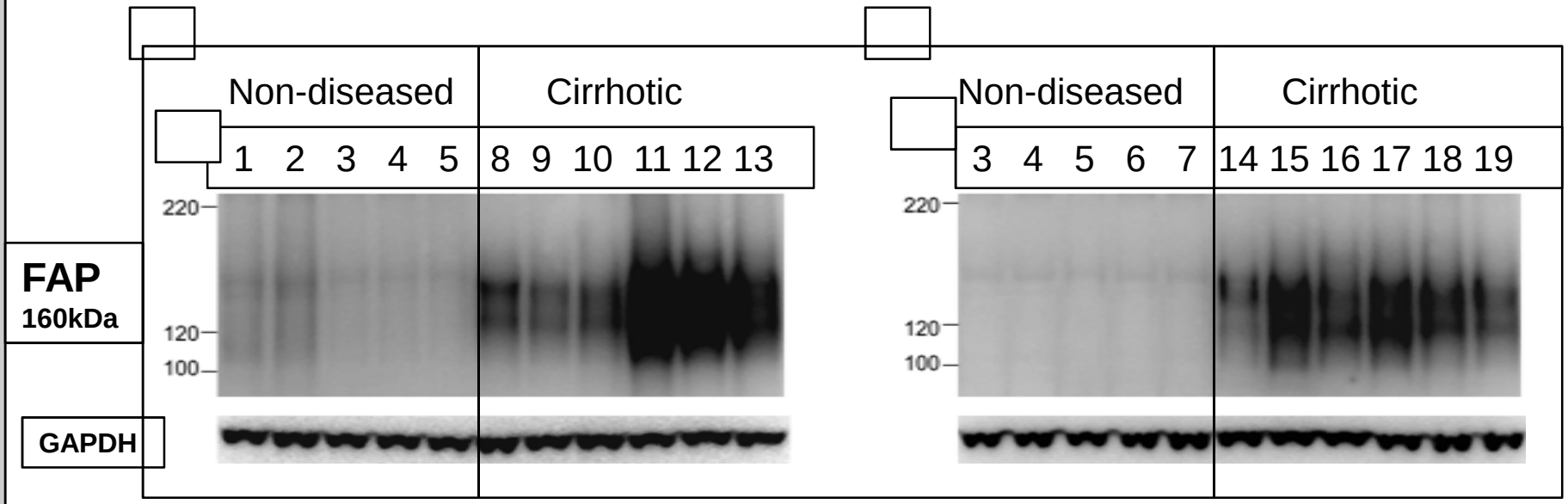
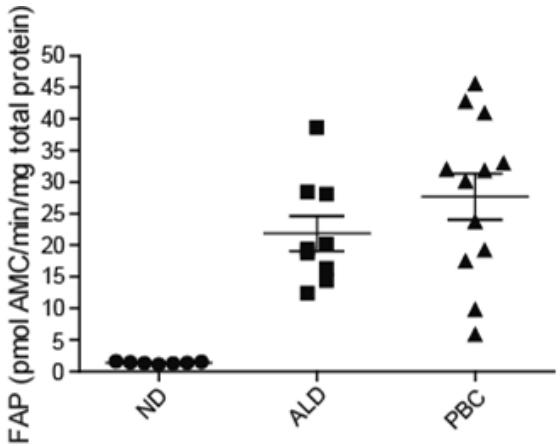
FAP is elevated in human cirrhosis

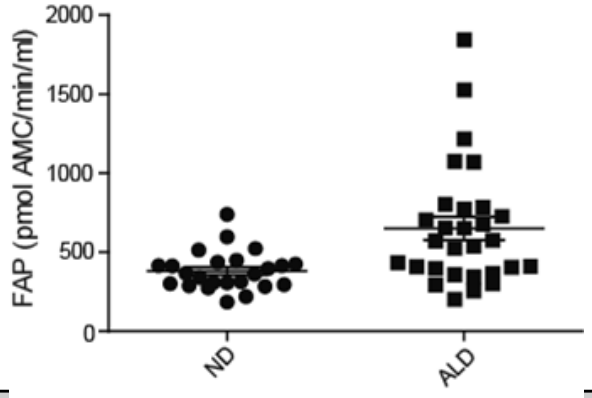
Immunoblot on human liver: Anti-human FAP



FAP enzyme activity in livers



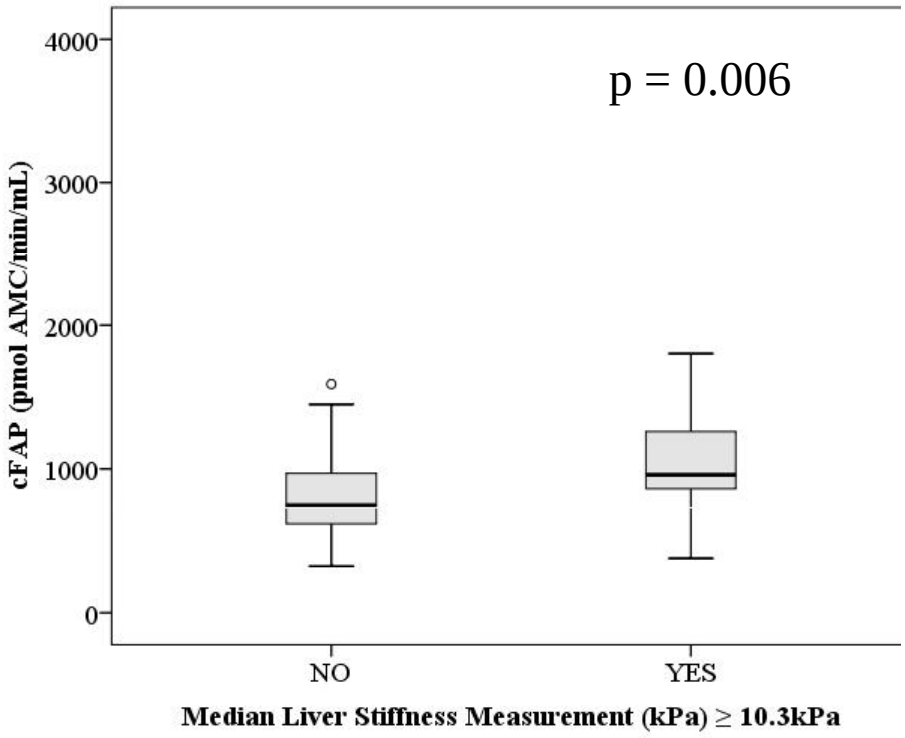
FAP enzyme activity in sera



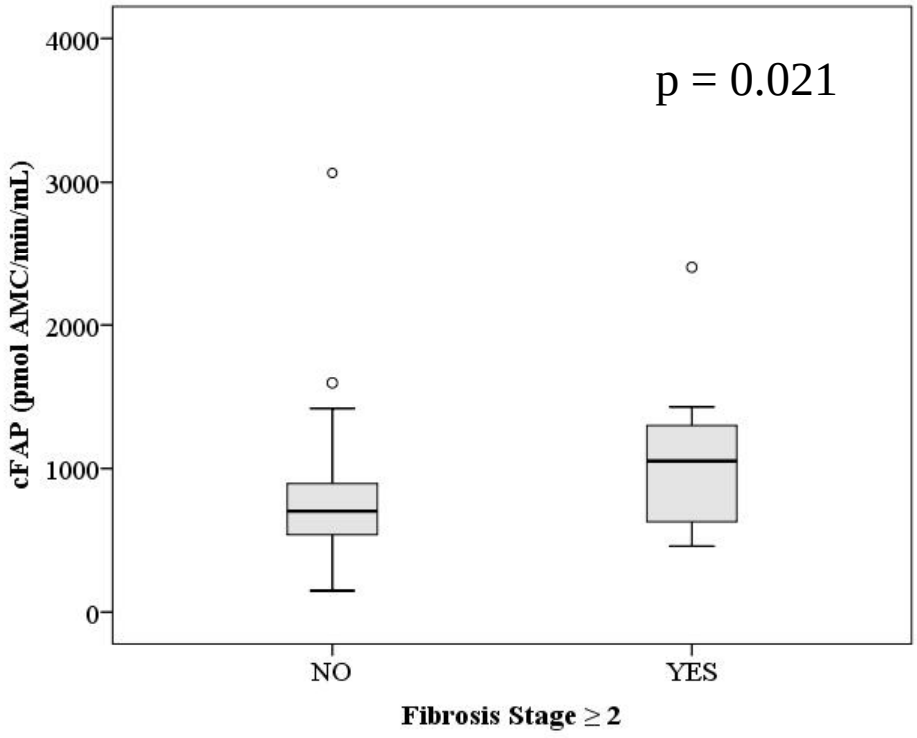
Keane, Yao, *et al,* ….. Bachovchin , Twigg, Gorrell *FEBS OpenBio* 2014

Relationship between clinically significant liver fibrosis and cFAP

Cohort 1, diabetes



Cohort 2, obesity

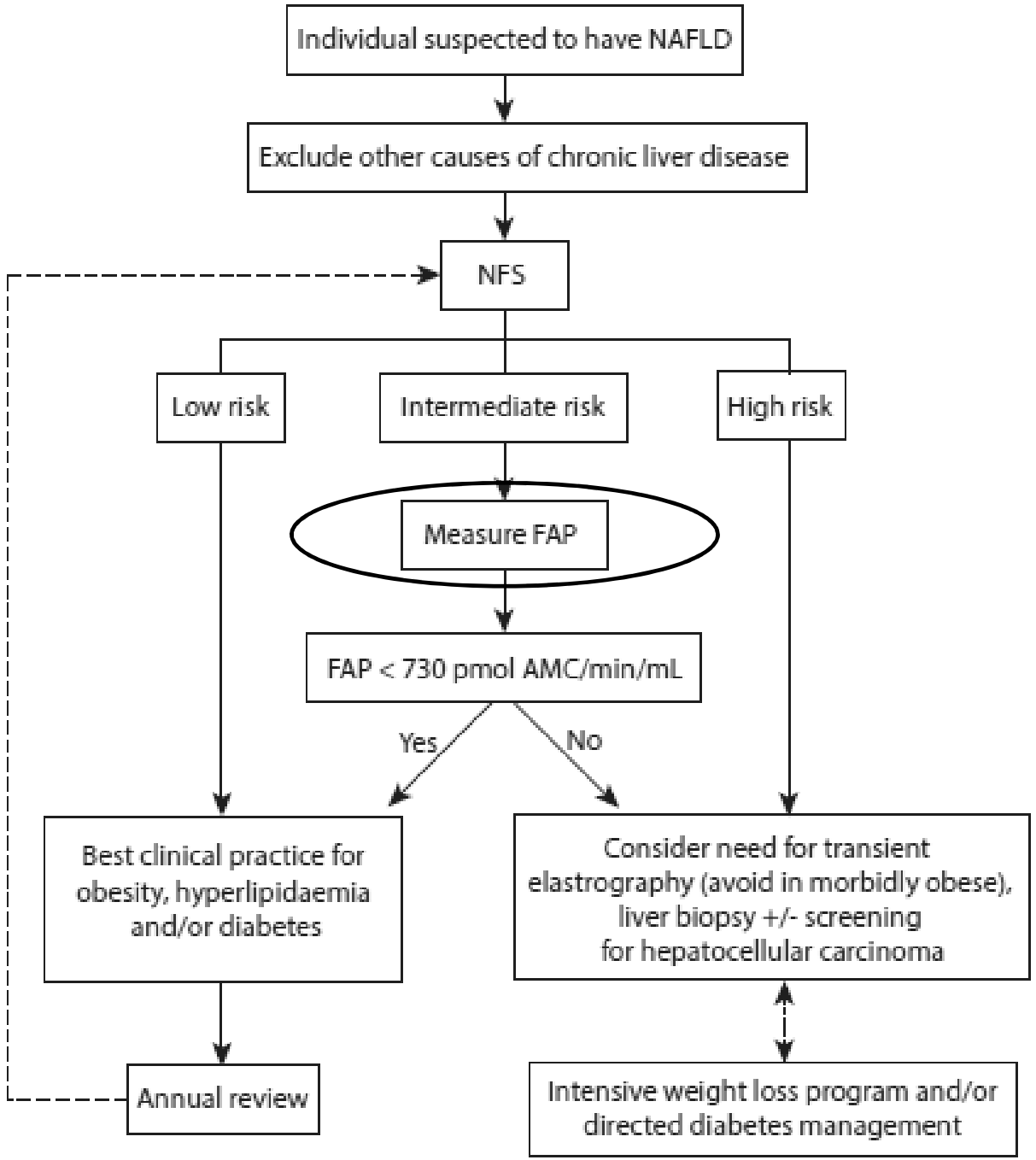


K. Williams, F. Keane, M. Gorrell, A. Zekry, S. Twigg

Suggested clinical use of cFAP:

<u>40% of Pts</u>:
Intermediate NFS
low cFAP
No fibrosis

K. Williams
S. Twigg



Substrates in liver: potential biomarkers?

FAP:

- Alpha-2-antiplasmin
- Collagen I

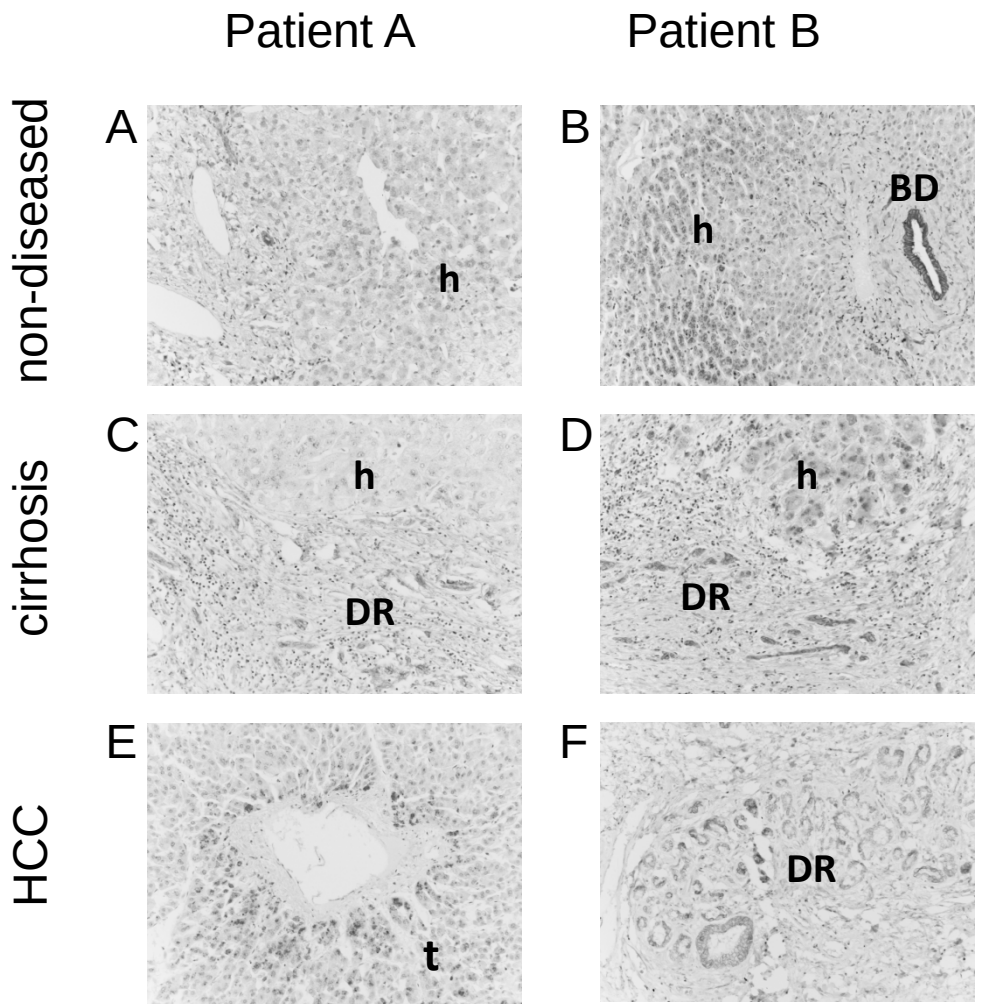
DPP4 and FAP:

- Neuropeptide Y

DPP4:

- CXCL9
- CXCL10
- CXCL12

NPY [neuropeptide Y] expression in human liver



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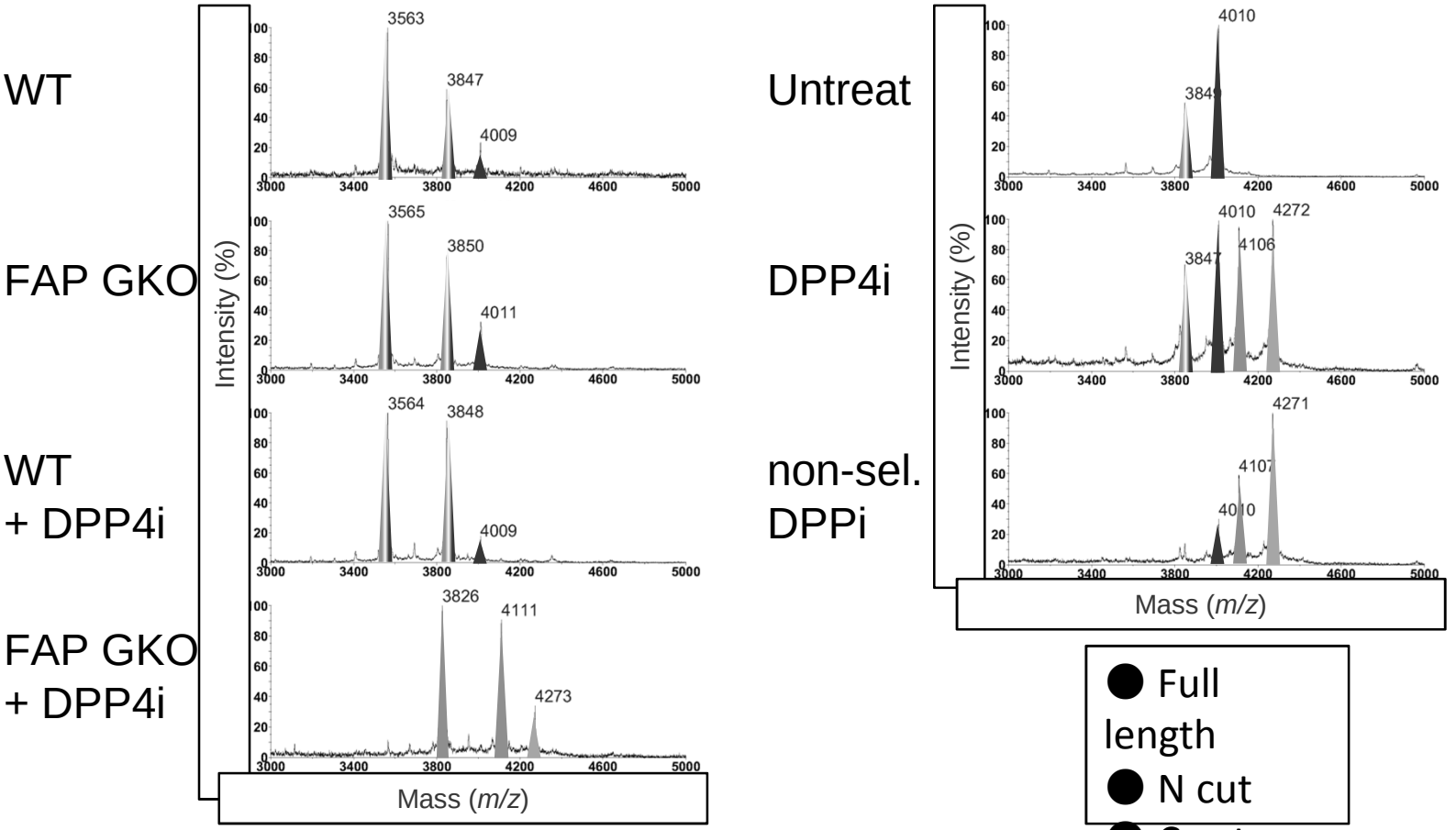
Neuropeptide Y

Mouse

Human



PF Wong

Neuropeptide Y with carboxypeptidase inhibition

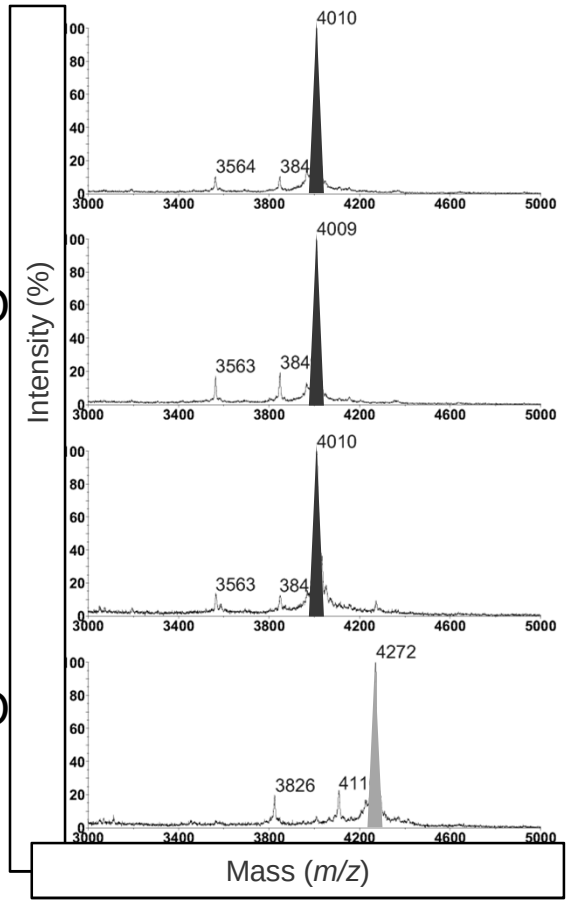
Mouse

WT

FAP GKO

WT + DPP4i

FAP GKO + DPP4i

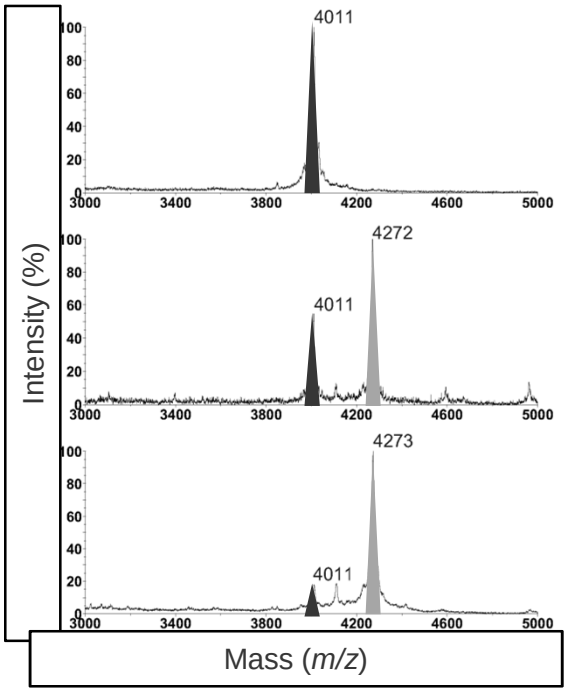


Human

no DPPi

DPP4i

non-sel. DPPi



● Full length

● N cut

● C cut

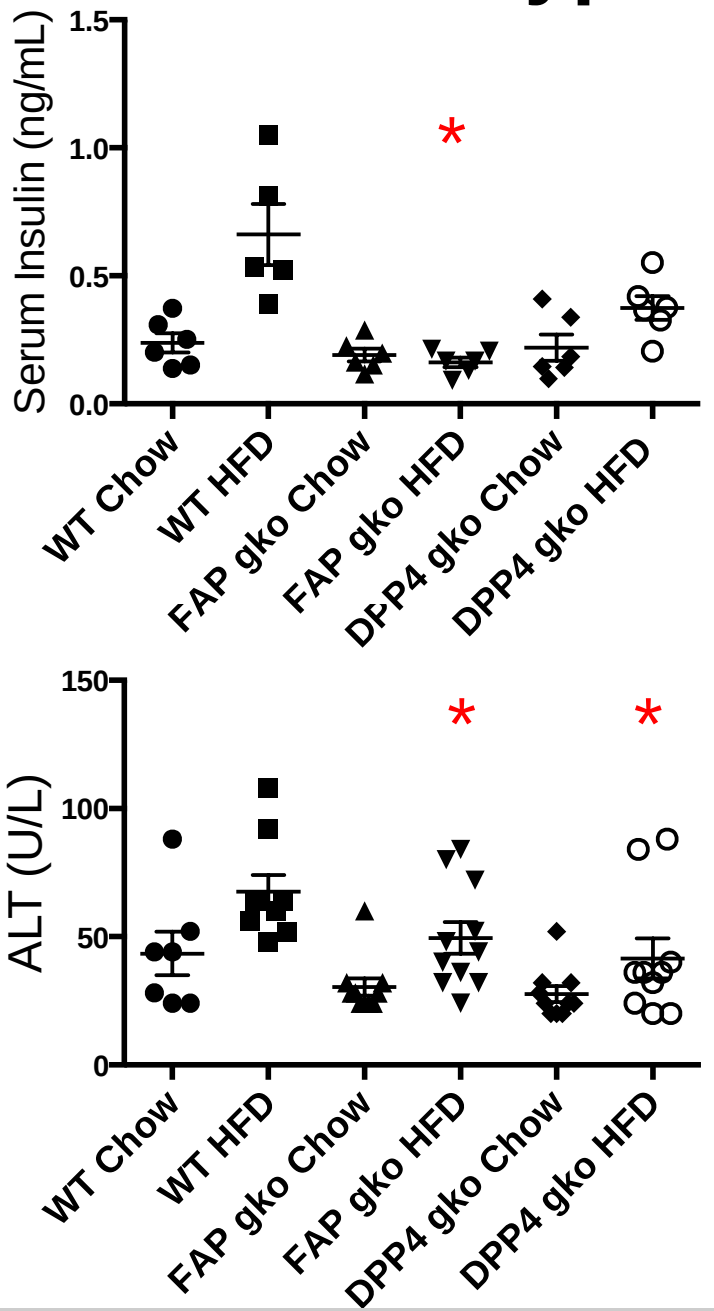
PF Wong

FAP gene knockout [gko] Mouse Phenotype:

Humans lacking active FAP have no adverse effects [Osborne, …Gorrell 2014 *BBA Proteins*]

FAP gko mouse:

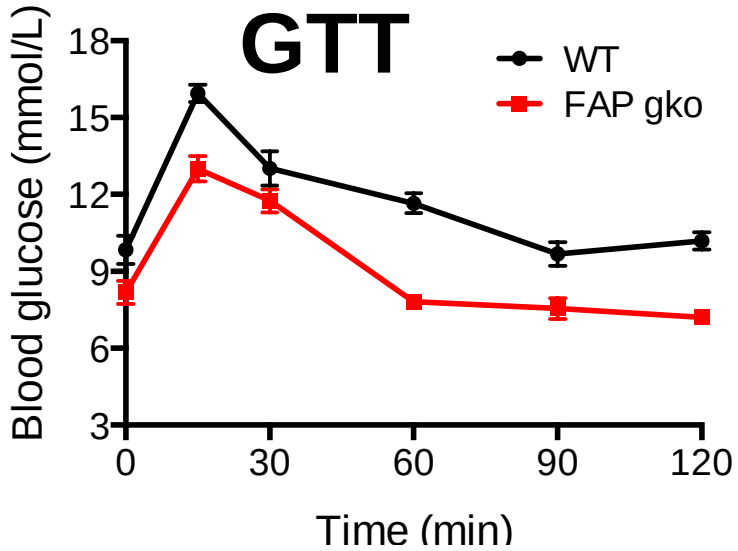
- Healthy and viable
- In liver fibrosis models:
  - Less fibrosis
  - Less inflammation
- In high fat diet (HFD) induced obesity (DIO) model:
  - Less liver lipid
  - Greater glucose tolerance
  - Less insulin resistance [HOMA-IR]
  - Less liver injury[ALT]

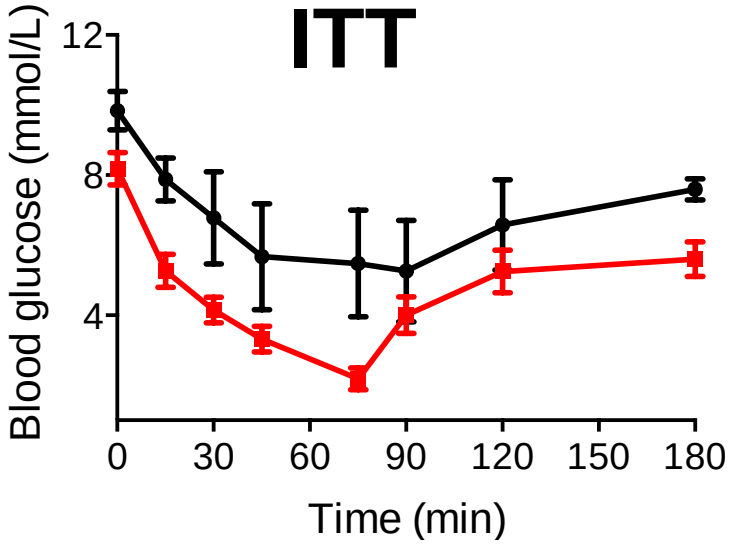


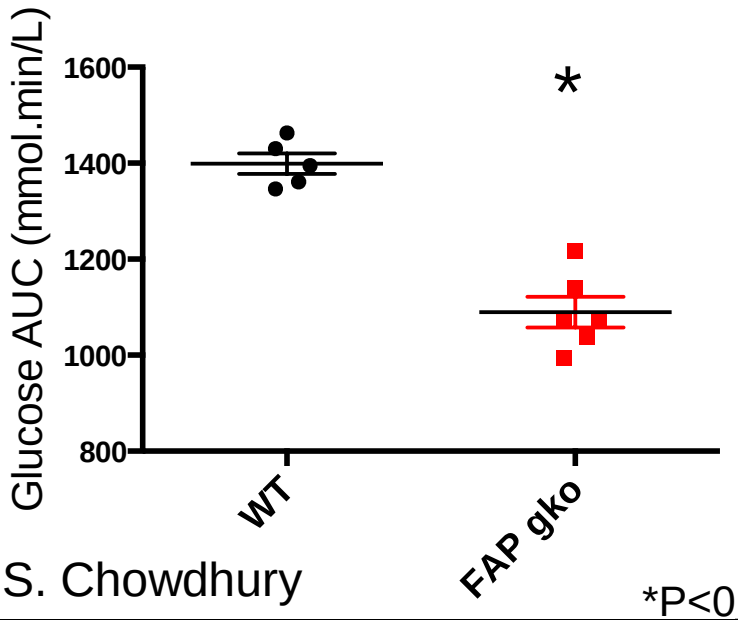
*p<0.05 compared to WT HFD

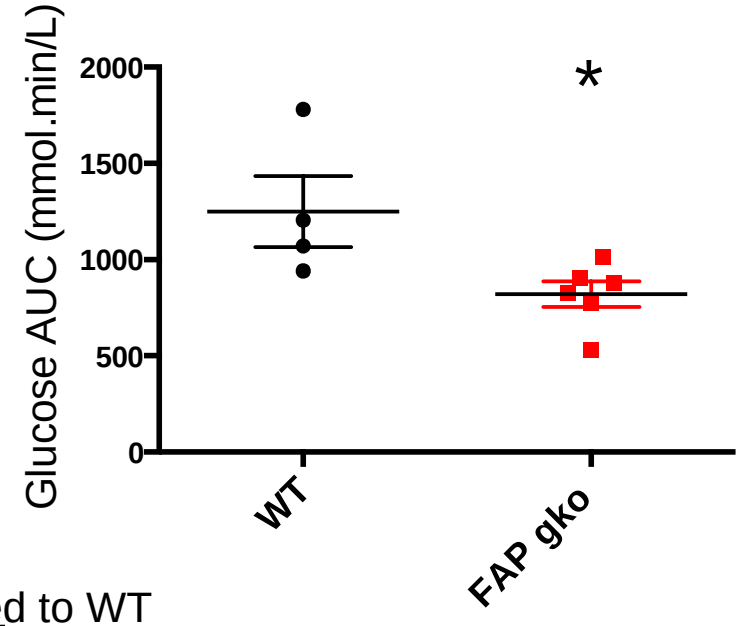
S. Chowdhury

FAP gko mouse: improved glucose tolerance and insulin tolerance at 20 weeks DIO









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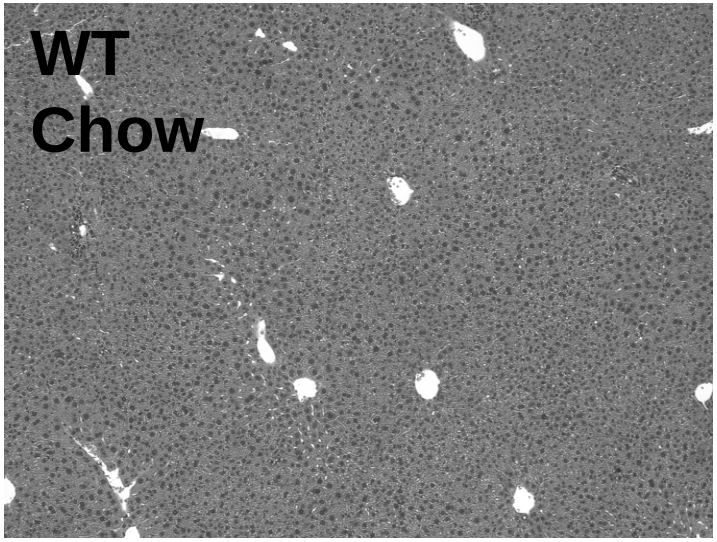
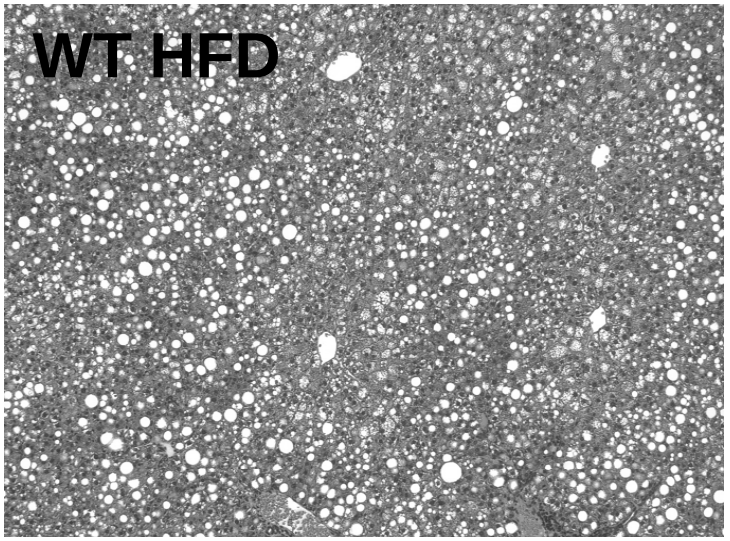
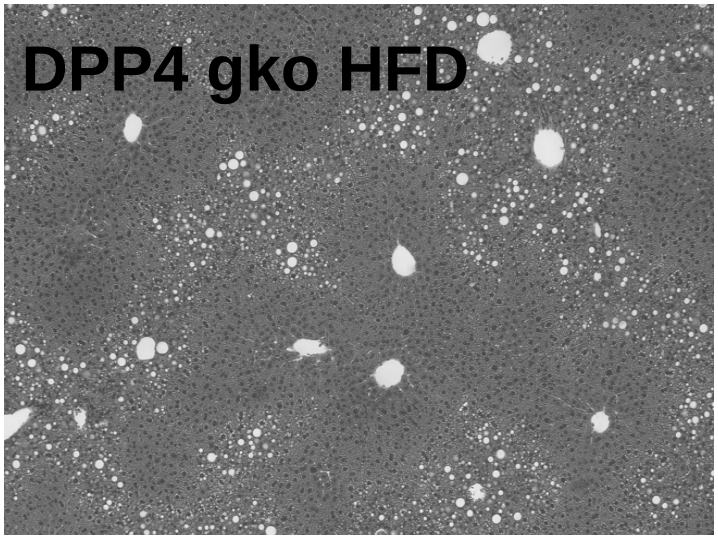
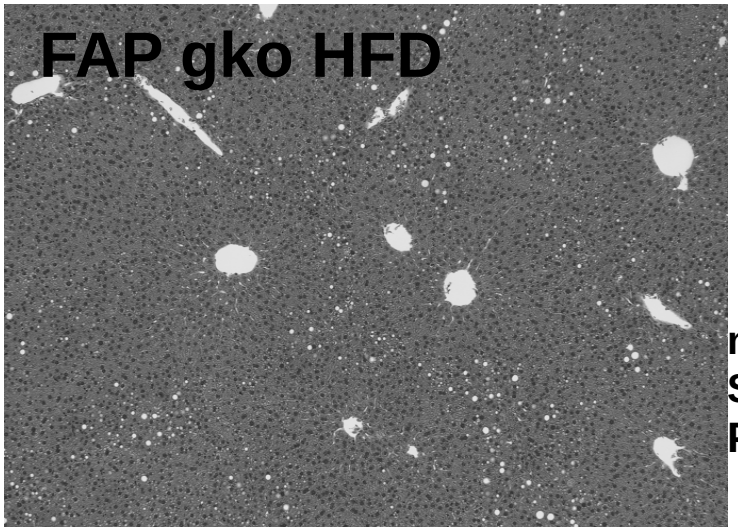
*P<0.05 compared to WT

FAP gko mice 20 weeks DIO: Less severe liver histology (H&E)









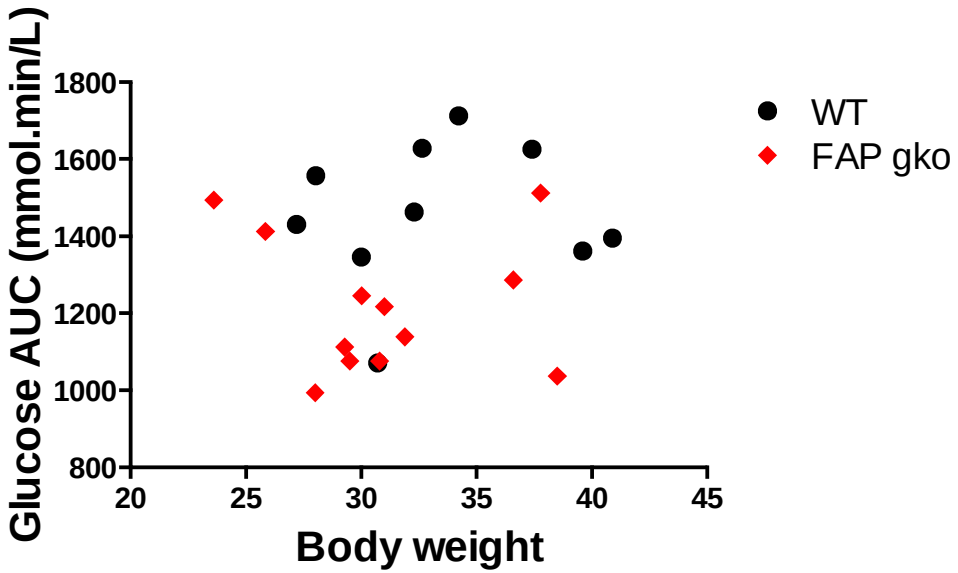
FAPgko qPCR

FoxO1
ApoC3
PPAR g
TNF

no change
SREBP1c
PPARα

S Chowdhury, M Gall, S Song

Improved oGTT is unrelated to body weight

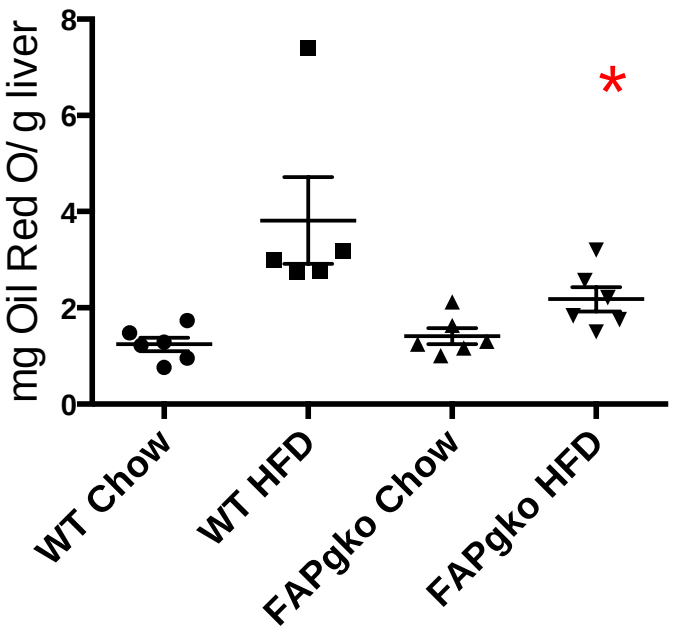


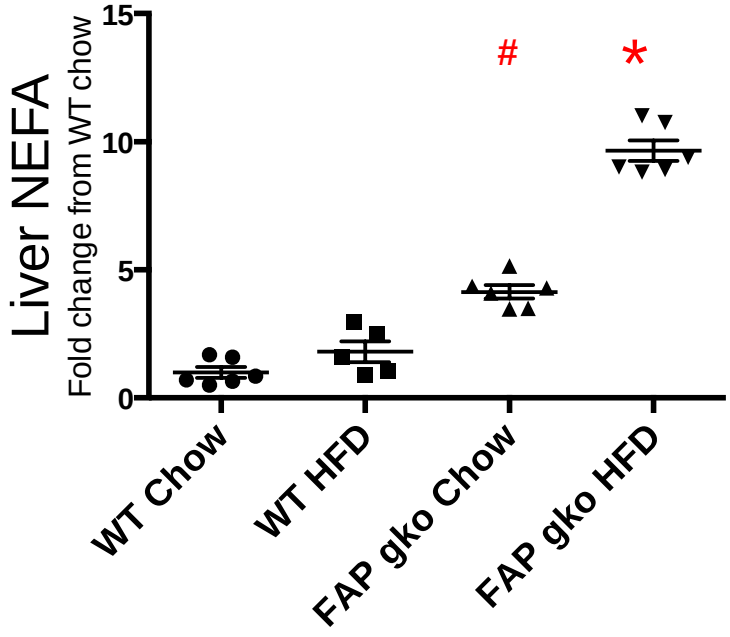
20 weeks DIO.
n = 10-12 per group

Level of intact GLP1 unchanged in FAPgko

S. Chowdhury

Mechanisms in FAP gko liver:
Less lipid: Non-esterified fatty acids elevated [consistent with increased fat burning]
FA import (CD36) and lipogenic (GK) genes down





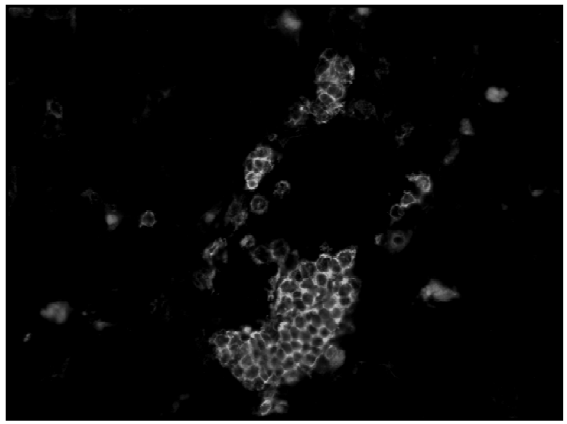
# p < 0.05 compared to WT Chow

* p < 0.05 compared to WT HFD

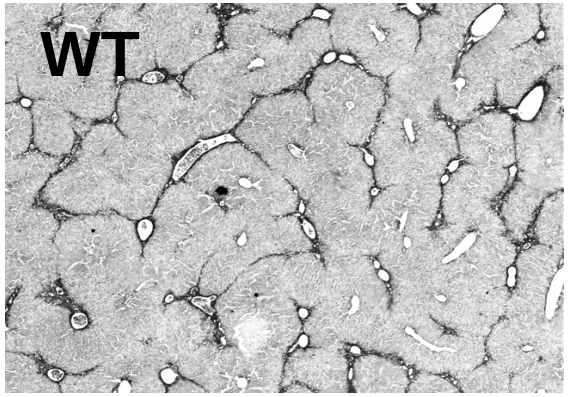
Lower levels of CD36 and glucokinase

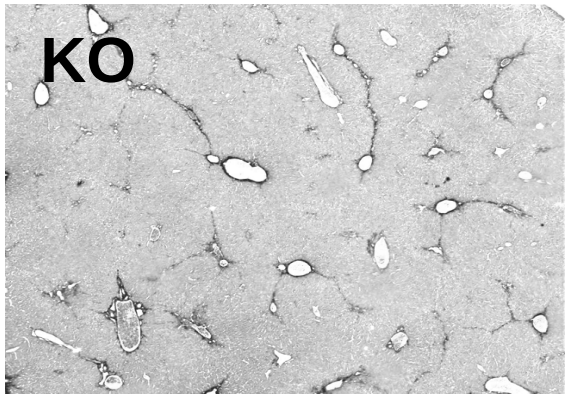
FAP gko Mouse Phenotype

- Liver fibrosis CCl_4 model:
  - Less fibrosis
  - Less inflammation [B cell clusters]



B cell clusters in liver





Sirius red stain of crosslinked collagen

XM Wang

Discussion:

- **DPP4** inhibition lessens steatosis and possibly fibrosis.
- **DPP4** may be a liver damage biomarker; possibly of apoptosis.

- **FAP** is fibrosis associated; highly upregulated in activated mesenchymal and stellate cells in liver.
- **FAP** gko mouse has less fibrosis and less steatosis, and improved glucose tolerance and insulin sensitivity.
- Mechanism may involve increased fat burning, less FA uptake, less lipogenesis.
- How FAP lowers insulin is not known [not GLP1]. [leptin?]
- Need to discover FAP substrates.
- Potential for dual blockade of DPP4 and FAP to improve glycaemic parameters.

- Potential for DPP4, FAP and/or their substrates to become biomarkers.

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Merck USA and MSD for MK626
Boehringer Ingelheim for FAP gko mouse
D Marguet for DPP4 gko mouse

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